methanol (9:1)] R_{VI} = 0.65, R_{IV} = 1.2. The nmr spectrum showed signals of six 3-acetyl protons at τ 7.90, 7.94, 7.94, 8.01, 8.01, and 8.03.

Anal. Calcd for $C_{24}H_{33}NO_{15}$: C, 50.08; H, 5.78. Found: C, 50.11; H, 5.64.

Periodate Oxidation of V and VIII.—After deacetylation of the ester groups of IV and VII with barium methoxide, the resulting products were subjected to periodate oxidation as described previously for the glucosamine analogs.[5] Compound IV consumed 2.2 mol, whereas its 3-*O* isomer reacted with 1.05 mol of the reagent.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranose (IX).—Opening of the anhydro ring in IV was effected by treating 0.57 g with acetic anhydride (15 ml), glacial acetic acid (6 ml), and concentrated sulfuric acid (0.10 ml) at 25–26° for 4 hr. Anhydrous sodium acetate (1 g) was then added, and the suspension was taken to dryness. The residue was extracted with chloroform; the extract was washed with water and evaporated *in vacuo*. Methylene chloride–ether (6:4) eluted from a silica gel G column 0.46 g (70%) of a homogenous substance. After crystallization from ethanol–water (8:2) it had mp 104–106°; $[\alpha]^{22}D$ +42.7°; tlc [benzene–methanol (9:1)] R_{IV} = 0.79. The nmr spectrum showed signals at τ 7.84, 7.90, 7.90, 7.94, 7.94, 8.01, 8.01, and 8.04 (eight acetyl groups).

Anal. Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80. Found: C, 49.54; H, 5.65.

4-*O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (X).—A solution of IX (0.180 g) in absolute methanol (20 ml) was treated with 1 *N* barium methoxide (0.1 ml) during 4 hr at 2°. The solution was neutralized by stirring with Dowex 50W-X, filtered, and taken to dryness. Crystallization from methanol–ether (1:1) yielded 85 mg (83.5%) of a hygroscopic powder: mp 148–150°; $[\alpha]^{28}D$ +55.5° (*c* 1, water); tlc [benzene–methanol (1:2)] $R_{lactose}$ = 0.76, R_{IX} = 0.45.

Anal. Calcd for $C_{14}H_{25}NO_{11}\cdot{}^1/_2H_2O$: C, 42.86; H, 6.68; N, 3.57. Found: C, 42.70; H, 6.72; N, 3.70.

This disaccharide was also obtained by hydrogenolysis of XIV.

1,2,4,6-Tetra-*O*-acetyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-α-D-galactopyranose (XI).—The ring opening in VII was carried out at 17–19°. Methylene chloride–ether (7:3) eluted 60% of the product, which was crystallized from ether–hexane: mp 96–97°; $[\alpha]^{22}D$ +59.4°; tlc [benzene–methanol (185:15)] R_{VII} = 0.85. The nmr spectrum was identical with that of IX.

Anal. Calcd for $C_{28}H_{39}NO_{18}$: C, 49.63; H, 5.80; N, 2.07. Found: C, 49.48; H, 5.84; N, 2.20.

3-*O*-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-D-galactopyranose (XII).—The free disaccharide was obtained in 65% yield as described for X, after precipitation from methanol–ether and crystallization from 2-propanol: mp 163–165°; $[\alpha]^{23}D$ +56° (*c* 1, water); tlc [benzene–methanol (4:6)] $R_{lactose}$ = 0.75, R_X = 0.95.

Anal. Calcd for $C_{14}H_{25}NO_{11}$: C, 43.86; H, 6.57. Found: C, 43.31; H, 7.07.

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-dichloroacetamido-β-D-galactopyranosyl)-α-D-galactopyranose (XIII).—The glycoside III (200 mg) was dissolved in a mixture of acetic anhydride (7 ml), acetic acid (3 ml), and concentrated sulfuric acid (0.05 ml) and kept for 3 hr at 50°. Sodium acetate (200 mg) was added and the mixture was evaporated. The residue was taken up in chloroform (200 ml) which was washed several times with water. The chloroform solution was evaporated, and the residue was crystallized from alcohol–water (9:1): yield 200 mg (84.5%); mp 112°; $[\alpha]^{30}D$ +46.0°; tlc [benzene–methanol (8:2)] R_{III} = 1.26. The nmr spectrum showed signals of 15 aromatic protons, 12 acetoxy protons, and one N-dichloroacetyl proton.

Compound XIV, resulting from the catalytic deacetylation of XIII, was not isolated, but was converted into X by hydrogenolysis as described previously.[6]

Registry No.—III, 22176-21-2; IV, 22176-22-3; VI, 22176-23-4; VII, 22176-24-5; IX, 22176-25-6; X, 22176-26-7; XI, 22212-29-9; XII, 22176-27-8; XIII, 22176-28-9.

Nucleosides. LXIII. Synthetic Studies on Nucleoside Antibiotics. 3. Total Synthesis of 1-(4-Amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine, the Nucleoside Moiety of Gougerotin[1]

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Received June 20, 1969

The total synthesis of C-substance (the nucleoside product obtained from the antibiotic Gougerotin) from methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-galactoside (**1**) is described. 1-(4-Azido-4-deoxy-β-D-glucopyranosyl)-cytosine (**6**) was prepared by condensation of 4-*O*-mesyl-tri-*O*-benzoyl-β-D-galactosyl bromide (**2**, obtained from **1**) with N^4-acetylcytosine in the presence of mercuric cyanide in nitromethane followed by treatment with sodium azide and subsequent deacylation. Reduction of the azido derivative (**6**) afforded 1-(4-amino-4-deoxy-β-D-glucosyl)cytosine (**7**) which was selectively 4′-N-acetylated to **9** and peracetylated to the pentaacetate (**8**). Tritylation of **6** followed by benzoylation and detritylation gave 1-(4-azido-2,3-di-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl)-N^4-benzoylcytosine (**13**). Oxidation of **13** with chromic anhydride in wet pyridine–acetic acid afforded, after debenzoylation, 1-(4-azido-4-deoxy-β-D-glycopyranosyluronic acid)cytosine (**15**). Reduction of **15** yielded 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine, identical with C-substance obtained by acid hydrolysis of Gougerotin. Gougerotin-derived C-substance was converted to nucleosides **8** and **9**, which were identical with those obtained by chemical synthesis from **1**.

The nucleoside antibiotic Gougerotin, isolated by Kanzaki, *et al.*,[2] from *Streptomyces gougerotii*, inhibits protein biosynthesis by preventing the transfer of amino acids from amino acyl-tRNA to protein.[3] Details of the mechanism of this inhibition have been studied,[4] and in a recent report[5] it was shown that Gougerotin acts on the 50S ribosomal subunit and

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant CA 08748).

(2) T. Kanzaki, E. Higashide, H. Yamamoto, M. Shibata, K. Nakazawa, H. Iwasaki, T. Takewaka, and A. Miyake, *J. Antibiot.* (Tokyo), *Ser. A*, **15**, 93 (1962).

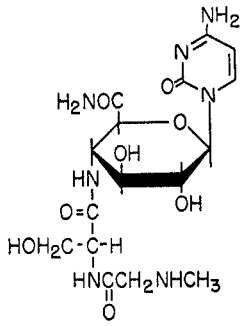
(3) J. M. Clark, Jr., and J. K. Gunther, *Biochim. Biophys. Acta*, **76**, 636 (1963); J. M. Clark, Jr., and A. Y. Chang, *J. Biol. Chem.*, **240**, 4734 (1965); H. Sinohara and H. H. Sky-Peck, *Biochem. Biophys. Res. Commun.*, **18**, 98 (1965).

(4) S. R. Casjens and A. J. Morris, *Biochem. Biophys. Acta*, **108**, 677 (1965); I. H. Goldberg and K. Mitsugi, *Biochemistry*, **6**, 383 (1967); C. Coutsogeorgopoulos, *ibid.*, **6**, 1704 (1967); J. M. Clark, Jr., *Antibiotics*, **1**, 278 (1967); R. E. Monro and K. A. Marcker, *J. Mol. Biol.*, **25**, 347 (1967); R. E. Monor and D. Vazquez, *ibid.*, **28**, 161 (1967).

(5) A. E. Herner, I. H. Goldberg, and L. B. Cohen, *Biochemistry*, **8**, 1335 (1969).

stabilizes the bound *N*-acetyl-L-phenylalanyl-tRNA. It is reported[6] that Gougerotin also acts as an inhibitor of the growth of certain viruses.

These data strongly suggest that this antibiotic and related structures have potentialities as biochemicals useful for the elucidation of fine mechanisms involved in protein biosynthesis and for general chemotherapeutic investigation.[7] Therefore, a program directed toward the total synthesis of Gougerotin and related compounds was undertaken in our laboratory.



Gougerotin

Early degradative studies[8] showed that Gougerotin was hydrolyzed in 6 *N* hydrochloric acid to ammonia, D-serine, sarcosine, and a nucleoside derivative, C-substance. Subsequent studies[9] established 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine as the structure of C-substance and 1-(4-sarcosyl-D-seryl-amino-4-deoxy-β-D-glucopyranosyluronamide)cytosine as that for Gougerotin by chemical degradation and physico-chemical means. In this report we describe the chemical synthesis of C-substance and related nucleosides from D-galactose, and the identity of these synthetic compounds with products derived from Gougerotin. A preliminary report[10] on some parts of these syntheses has appeared.

Methyl α-D-galacotopyranoside[11] was converted to methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-β-D-galactoside (**1**) in two steps according to the method of Reist, *et al.*[12] Treatment of **1** with glacial acetic acid saturated with hydrogen bromide afforded the 1-bromo sugar **2** as a syrup (Scheme I). The nmr spectrum of this syrup showed it to be the α anomer, as indicated by the low-field doublet (δ 6.88, $J_{1,2}$ = 4.0 Hz). When the syrup **2** was condensed with N^4-acetylcytosine by the general nitromethane–mercuric cyanide procedure,[13] essentially pure, crystalline nucleoside **3** precipitated in high yield from the reaction mixture. The uv absorption spectrum of product **3** resembles that of 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-N^4-acetylcytosine,[9] confirming N^1 glycosylation. The large spacing of the low-field anomeric doublet in the nmr spectrum (δ 6.63, $J_{1',2'}$ = 9.0 Hz) established the glycosyl linkage to be of the β configuration. Treatment of nucleoside **3** with sodium azide in dimethylformamide afforded the azido derivative **4**; however, the yields were poor and inconsistent. These difficulties were overcome by the use of hexamethyl phosphorotriamide (HMPT), a solvent reported[14] to be superior to dimethylformamide in nucleophilic displacement reactions. The by-product of this reaction (**3** → **4**) was the deacetylated derivative **5**, which was isolated as an amorphous powder and reacetylated to **4**. Deacylation of **4** with sodium methoxide in methanol gave the unblocked azido nucleoside **6** as colorless crystals. Conversion of the azido derivative **6** to 1-(4-amino-4-deoxy-β-D-glucopyranosyl)cytosine (**7**) was accomplished by hydrogenation over palladium on charcoal. Peracetylation and N-acetylation of **7** afforded nucleosides **8** and **9**, respectively.

C-Substance was obtained from Gougerotin and converted in three steps according to Iwasaki[15] into 1-(4-acetamido-4-deoxy-β-D-glucopyranosyl)cytosine (**9**) and then peracetylated to **8**. The nucleosides thus obtained from the antibiotic were identical in all respects with compounds **8** and **9** synthesized as described above from D-galactose.

A logical approach to the synthesis of C-substance is either to condense a preformed derivative of 4-amino-4-deoxy-D-glucuronic acid with the appropriate cytosine derivative or to selectively oxidize nucleosides related to **7** at the 6′ position. Recently, we achieved the first chemical synthesis[16] of 4-amino-D-hexuronic acid derivatives and related compounds, including methyl (methyl 4-amino-4-deoxy-α-D-glucopyranosiduronate) and methyl (methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranosiduronate). Several attempts to prepare the necessary halogenose from the latter 4-azido derivative were unsuccessful, owing primarily to the relative instability of the methyl ester linkage (*vs.* the methyl glycosidic linkage) to brominolysis conditions. This approach from a preformed 4-azido-4-deoxyhexuronic acid derivative was therefore temporarily abandoned.[17]

Catalytic oxidation of several nucleosides in the presence of platinum had been reported.[18] However, nucleosides **6** and **9** resisted all attempts toward platinum-catalyzed oxidation to the corresponding uronic acid, even though the catalyst was active in our hands for the reported[18b] oxidation of 1-β-D-arabinofuranosylcytosine to its uronic acid derivative. With ruthenium

(6) L. Thiry, *J. Gen. Virol.*, **2**, 143 (1968).

(7) J. J. Fox, K. A. Watanabe, and A. Bloch, *Progr. Nucleic Acid Res. Mol. Biol.*, **5**, 251 (1966).

(8) H. Iwasaki, *Yakugaku Zasshi*, **82**, 1385 (1962).

(9) J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda, and E. B. Whipple, *Antimicrobial Agents Chemotherapy*, 518 (1964); J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Lett.*, 6029 (1968).

(10) K. A. Watanabe, M. P. Kotick, and J. J. Fox, *Chem. Pharm. Bull.* (Tokyo), **17**, 416 (1969).

(11) J. L. Frahn and J. A. Mills, *Aust. J. Chem.*, **18**, 1303 (1965). Commercially available from Phansteihl Laboratories, Inc. Waukegan, Ill.

(12) E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker, and L. Goodman, *J. Org. Chem.*, **30**, 2312 (1965).

(13) N. Yamaoka, K. Aso, and K. Matsuda, *ibid.*, **30**, 149 (1965); K. A. Watanabe and J. J. Fox, *J. Heterocycl. Chem.*, **6**, 109 (1969).

(14) J. J. Delpuech, *Tetrahedron Lett.*, 2111 (1965); Y. Ali and A. C. Richardson, *J. Chem. Soc., C*, 1764 (1968).

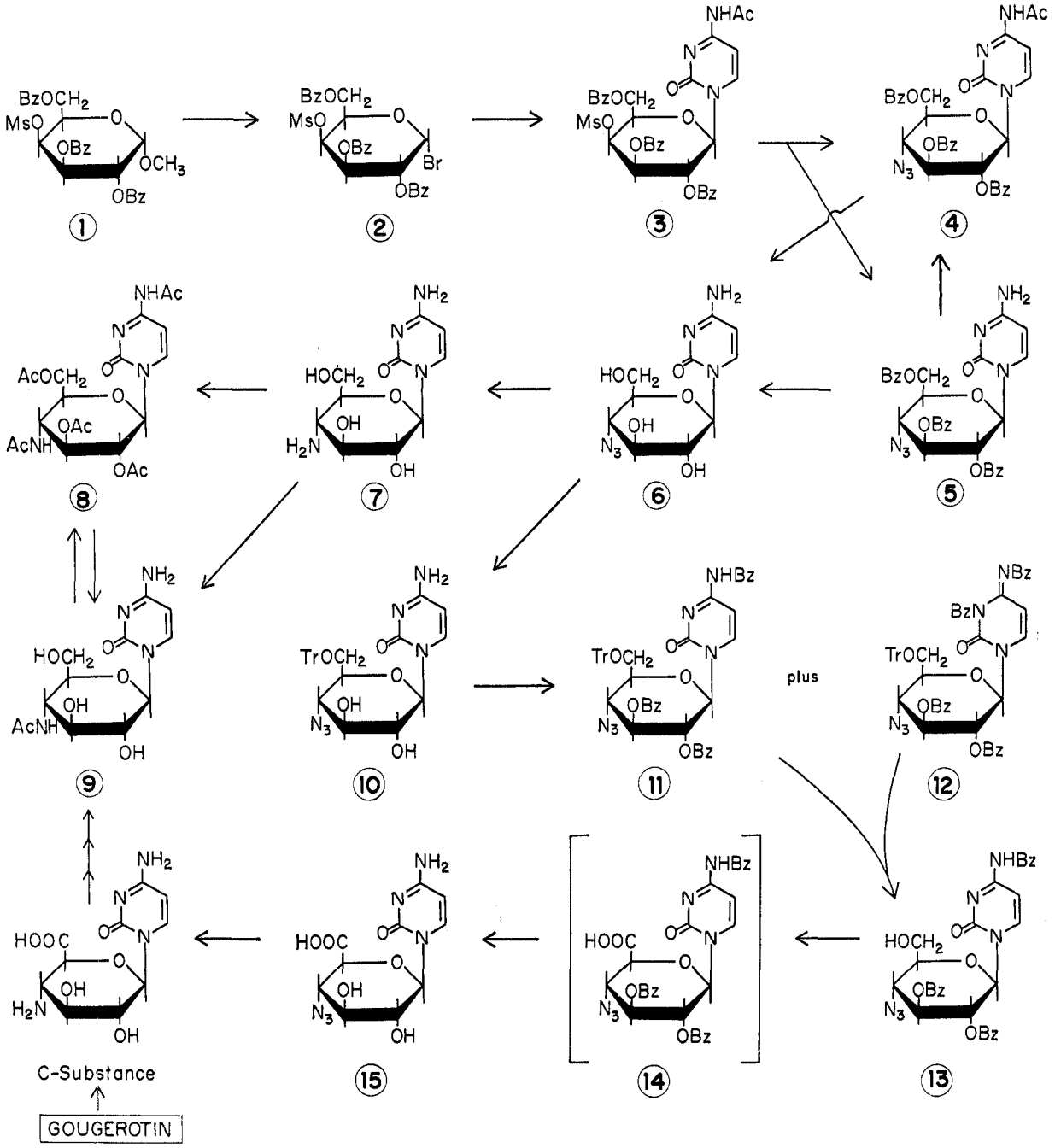
(15) H. Iwasaki, *Yakugaku Zasshi*, **82**, 1387 (1962).

(16) M. P. Kotick, R. S. Klein, K. A. Watanabe, and J. J. Fox, *Carbohyd. Res.*, in press.

(17) (a) It should be noted that 1-(β-D-glucopyranosyluronic acid)cytosine and -uracil have been synthesized[17b] by reaction of methyl tri-*O*-acetyl-D-glucopyranosyluronate bromide with 2,4-dialkoxypyrimidine. The synthesis of this bromo sugar was reported[17c] from methyl tetra-*O*-acetyl-D-glucopyranuronate. (b) T. Kishikawa, T. Yamazaki, and H. Yuki, *Chem. Pharm. Bull.* (Tokyo), **14**, 1354 (1966); (c) W. D. S. Bowering and T. E. Timell, *J. Amer. Chem. Soc.*, **82**, 2827 (1960).

(18) (a) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, and A. R. Todd, *J. Chem. Soc.*, 1149 (1963); (b) K. Imai and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **13**, 7 (1965); (c) K. C. Tsou, N. J. Santora, and E. E. Miller, *J. Med. Chem.*, **12**, 173 (1969).

Scheme I



tetroxide,[19] some reaction did occur; however, no product with a cytidine- or uridine-like spectrum could be obtained. Chemical oxidation of **6** or **9** with chromic anhydride–pyridine complex, a method which had been applied successfully to the oxidation of 2′-deoxycytidine to its corresponding uronic acid,[20] also failed; the reaction was accompanied by loss of selective absorption in the ultraviolet.

Because of the failures to oxidize **6** or **9** to their uronic acids with these and other oxidants, we undertook the synthesis of a nucleoside in which *only* the 6′-hydroxyl is unprotected.[21] Selective tritylation of **6** afforded the 6′-tritylate **10**. Benzoylation of **10** with 4 equiv of benzoyl chloride in pyridine at *ca.* 10° gave a mixture of the tri- and tetrabenzoates **11** and **12** in a ratio of 6:4, respectively. Even under milder conditions (−10°) using only 3.03 equiv of benzoyl chloride, the formation of small amounts of **12** was observed by tlc examination.[22] Detritylation of **11** with a catalytic

(19) L. M. Berkowitz and P. N. Rylander, *J. Amer. Chem. Soc.*, **80**, 6682 (1958); P. J. Beynon, P. M. Collins, and W. G. Overend, *Proc. Chem. Soc.*, 342 (1964); P. J. Beynon, P. M. Collins, P. T. Doganges, and W. G. Overend, *J. Chem. Soc.*, 1131 (1966); V. M. Parikh and J. K. N. Jones, *Can. J. Chem.*, **43**, 3452 (1965); R. F. Nutt, M. J. Dickinson, F. W. Holly, and E. Walton, *J. Org. Chem.*, **33**, 1789 (1968).

(20) A. S. Jones, A. R. Williamson, and M. Winkley, *Carbohyd. Res.*, **1**, 187 (1965).

(21) (a) Such a derivative (**13**) should at least be applicable to oxidation by the Pfitzner–Moffatt method[21b] to the corresponding aldehyde. (b) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **85**, 3027 (1963); **87**, 5661 (1965); K. Onodera, S. Hirano, and N. Kashimura, *ibid.*, **87**, 4651 (1965); K. Sowa and G. H. S. Thomas, *Can. J. Chem.*, **44**, 836 (1966).

(22) (a) It should be noted that the formation of pentabenzoylcytidine (analogous to **12**) by benzoylation of cytidine with a large excess of benzoyl chloride under *vigorous* conditions (100°) had been observed by Brown, Todd, and Varadarajan.[22b] When the reaction was conducted with 12 equiv of benzoyl chloride at room temperature, they isolated tetrabenzoylcytidine in high yield. It would appear that with nucleoside **10**, dibenzoylation of the aglycon occurs with greater ease than with cytidine. (b) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2384 (1956).

amount of hydrochloric acid in ethanol–chloroform afforded the crystalline 5′-hydroxymethyl nucleoside **13**. Attempts to oxidize **13** with chromic anhydride using a variety of reported conditions[23] were unsuccessful. It was found, however, that oxidation of **13** occurred smoothly when it was treated with chromic anhydride in a 1:1 mixture of pyridine–acetic acid containing a small amount of water.[24] The protected uronic acid nucleoside **14** thus obtained was isolated as an amorphous powder, and without further purification was debenzoylated with sodium methoxide in methanol. A mixture was obtained which contained only two components, of which the major product (*ca.* 55% overall yield from **13**) was the desired nucleoside **15**. The minor component was starting material **6**, thus indicating that the oxidation step was incomplete. When compound **12** was treated with a small amount of hydrochloric acid in a 1:1 mixture of chloroform and dioxane, followed by chromic anhydride oxidation and debenzoylation, two acidic products were detected by paper electrophoretic examination. One of the products showed mobility on paper electrophoresis and uv characteristics identical with those of authentic **15**. The faster moving component possessed a uridine-like uv absorption spectra, indicating that in the course of this reaction some hydrolytic deamination had occurred.

Hydrogenation of **15** over palladium on charcoal gave C-substance in quantitative yield. The C-substance thus obtained was identical in all respects (uv, ir, $[\alpha]$D, paper electrophoresis) with that derived by acid hydrolysis of Gougerotin. It may be argued that in the conversion of **13** to C-substance, epimerization at C-5′ may have occurred leading to a "C-substance" of the L-ido configuration. Such a C-5′ epimerization might have been expected on the basis of the reported[25] epimerization of alduronic acids at C-5 which occurred when these uronic acids were heated in aqueous solution, though others[26] later failed to observe such an epimerization. However, we have demonstrated previously[16] that the configuration at C-5 was unchanged during conversion of methyl 4-azido-di-*O*-benzoyl-4-deoxy-α-D-glucopyranoside into methyl (methyl 4-amino-4-deoxy-α-D-glucopyranosiduronate) *via* oxidation, esterification, debenzoylation, and reduction. These reactions are analogous to those involved in the conversion of **13** to C-substance, which would rule out C-5′ epimerization. The above observations are important, since none of the derivatives of C-substance gave first-order nmr spectra, thus mitigating against the use of nmr for configurational assignments.

The total synthesis of C-substance described herein offers conclusive proof that the structure of this compound is 1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine and constitutes a first synthesis of a aminohexuronic acid nucleoside. Studies directed toward the chemical synthesis of Gougerotin are underway in our laboratory.

(23) L. F. Fieser, *J. Amer. Chem. Soc.*, **70**, 3237 (1948); G. I. Poos, G. E. Arth, R. E. Beyler, and C. H. Sarrett, *ibid.*, **75**, 422 (1953); R. H. Cornforth, J. W. Cornforth, and G. Papjak, *Tetrahedron*, **18**, 1351 (1962).

(24) This reagent mixture [pyridine–acetic acid (1:1) containing a small amount of water] should find wide usage in the chromic anhydride oxidations of carbohydrates under mild conditions.

(25) F. G. Fischer and H. Schmidt, *Chem. Ber.*, **92**, 2184 (1959).

(26) M. B. Perry and R. K. Hulyalkar, *Can. J. Biochem.*, **43**, 573 (1965).

Experimental Section[27]

1-(2,3,6-Tri-*O*-benzoyl-4-*O*-methanesulfonyl-β-D-galactosyl)-N^4-acetylcytosine (3).—Compound **1** (104 g, 0.178 mol) was suspended in glacial acetic acid (800 ml, saturated with HBr at 0°). The suspension was shaken at room temperature until a clear solution was obtained (14–24 hr). The mixture was diluted with methylene chloride (1200 ml) and extracted successively with ice-cold water (two 1-l. portions), cold sodium bicarbonate solution (two 1-l. portions), and water (two 1-l. portions). The organic layer was dried over sodium sulfate, filtered, and condensed to *ca.* 200 ml *in vacuo* below 40°.

A mixture of nitromethane (3 l.), mercuric cyanide (45 g, 0.18 mol), and N^4-acetylcytosine (13.7 g, 0.089 mol) was refluxed and about 150 ml of the solvent was removed by azeotropic distillation. To the stirred, refluxing mixture was added the previously prepared methylene chloride solution of the halogenase dropwise over a period of *ca.* 10 min. After 3.5 hr the reaction was cooled to room temperature and the clear, orange-colored solution was allowed to stand overnight. Colorless crystals deposited, which were filtered off and washed with a small amount of nitromethane, 30% aqueous potassium iodide, water, and a small amount of cold ethanol. The colorless needles thus obtained (53.0 g, 84%) had mp 259–263° dec; $[\alpha]^{25}$D +38° (*c* 1.1, DMF); uv λ_{max}^{EtOH} 300 (ε 6000), 282 (ε 6600), and 232 mμ (ε 45,000); λ_{min}^{EtOH} 288 (ε 5500), 278 (ε 6300), and 277 mμ (ε 24,800); ir λ_{max}^{KBr} 3.0 (NH), 5.8 (C=O benzoyl), 5.89 (*N*-acetyl), 6.02, 6.14 (pyrimidine), 7.90 (COC benzoyl), 8.5 (sulfonate), 9.1 (COC sugar), and 14.0 μ (phenyl); nmr (DMSO-d_6) δ 6.63 (d, 17, aromatic H, H-1′), 3.42 (mesyl H), and 2.08 (*N*-acetyl H).

Anal. Calcd for $C_{34}H_{31}O_{12}N_3S$: C, 57.87; H, 4.40; N, 5.96; S, 4.54. Found: C, 58.08; H, 4.30; N, 5.94; S, 4.70.

The nitromethane mother liquor was evaporated to dryness *in vacuo*. The residual syrup was dissolved in a minimum amount of acetone and the solution was kept overnight at 4°. A further quantity (7.0 g, 11.5%) of compound **3** separated as colorless needles (mp 258–262° dec) for a total yield of 95.5%.

1-(4-Azido-2,3,6-tri-*O*-benzoyl-4-deoxy-β-D-glucosyl)-N^4-acetylcytosine (4).—A mixture of **3** (7.05 g), sodium azide (1.32 g), and HMPT (35 ml) was stirred for 4 hr at 80° and then diluted with an ice–water mixture (350 ml). The precipitate was filtered, washed with water, and dried. The dried precipitate was suspended in acetonitrile and refluxed for several minutes, during which time the precipitate changed into slightly yellowish fine needles: yield 4.94 g (76%); mp 240–242°; $[\alpha]^{27}$D +48° (*c* 1.2, DMF); uv λ_{max}^{EtOH} 300 (ε 6100), 282 (ε 6800), and 232 mμ (ε 38,800); λ_{min}^{EtOH} 288 (ε 5600), 278 (ε 6100), and 227 mμ (ε 22,800); ir λ_{max}^{KBr} 3.0 (NH), 4.70 (N_3), 5.8 (C=O benzoyl), 5.98 (*N*-acetyl), 6.02, 6.14 (pyrimidine), 7.92 (COC benzoyl), 9.2 (COC sugar), and 14.1 μ (phenyl).

Anal. Calcd for $C_{33}H_{28}O_9N_6$: C, 60.74; H, 4.32; N, 12.87. Found: C, 60.52; H, 4.16; N, 12.69.

In large-scale preparations (50 g of **3**), a longer reaction time (12 hr) was required and the yields were consistently lower. The crude mass obtained after washing the collected precipitate with water was crystallized from acetonitrile to give a *ca.* 50% (23–26 g) yield of **4**. Evaporation of the mother liquor gave an amorphous powder which was washed well with water and dried *in vacuo* (*ca.* 11 g). Column chromatography of a portion of this residue (3.0 g) on silica gel G (300 g, eluent 10:1 chloroform–methanol) yielded, after elution of **4**, nucleoside **5** (1.33 g), which could not be crystallized. The nmr and ir spectra showed the absence of acetyl and mesyl groups and the ir spectrum indicated the presence of an azido function in the product.

Anal. Calcd for $C_{31}H_{26}N_6O_8$: C, 60.98; H, 4.29; N, 13.77. Found: C, 60.41; H, 4.22; N, 13.44.

Compound **5** (500 mg) was treated with acetic anhydride (1 ml) in pyridine (5 ml) at room temperature for 90 min. Compound **4** (mp 245–247°, 474 mg, 89%) was obtained, which was identical with **4** described previously (nmr, ir, and mixture melting point).

1-(4-Azido-4-deoxy-β-D-glucopyranosyl)cytosine (6).—To a suspension of **4** (50 g) in methanol (500 ml) was added sodium

(27) Melting points were determined on a Thomas–Hoover apparatus (capillary method) and are corrected. The ultraviolet spectra were determined on a Cary Model 15 spectrophotometer; the nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer using tetramethylsilane as internal reference. Microanalyses were performed by Spang Microanalytical Laboratories, Ann Arbor, Mich., and by Galbraith Laboratories, Inc., Knoxville, Tenn.

methoxide (prepared by dissolving 100 mg of sodium in 20 ml of methanol) and the mixture was stirred overnight at room temperature. Precipitated **6** (14.65 g, mp 236–237° dec) was filtered and the filtrate, after neutralization with Dowex 50 (H^+), was concentrated to *ca.* 150 ml. A second crop of **6** (2.35 g, mp 236–237° dec) was obtained. Finally, the filtrate was evaporated to dryness and the residue (6.70 g) was refluxed with methanol (25 ml) for 15 min. After the mixture was cooled, a further amount of **6** (4.30 g, mp 234–235° dec) was obtained. The total yield of **6** was 21.2 g (92%): $[\alpha]^{25}D$ +58° (*c* 1.2, H_2O); uv $\lambda_{max}^{pH\ 14}$ 268 mμ (ε 9800); $\lambda_{max}^{pH\ 14}$ 253 mμ (ε 8600); $\lambda_{max}^{pH\ 6.8}$ 267 (ε 8600), 235 (ε 8100), and 198 mμ (ε 21,000); $\lambda_{min}^{pH\ 6.8}$ 250 (ε 7400), and 225 mμ (ε 7800); $\lambda_{max}^{pH\ 1}$ 276 mμ (ε 12,700); $\lambda_{min}^{pH\ 1}$ 240 mμ (ε 2500); ir λ_{max}^{KBr} 2.86 (OH), 3.06 (NH), 4.69 (N_3), 6.02, 6.20 (pyrimidine), 9.25 and 9.43 μ (COC sugar). The product analyzed best for a hemimethanolate.

Anal. Calcd for $C_{10}H_{14}N_6O_5 \cdot {}^1/_2CH_3OH$: C, 40.13; H, 5.09; N, 26.75. Found: C, 40.77; H, 4.70; N, 26.97.

Debenzoylation of compound **5** by a similar manner afforded **6**.

1-(4-Amino-4-deoxy-β-D-glucopyranosyl)cytosine (7).—Compound **6** (1.53 g) in water (70 ml) was hydrogenated over 5% palladium on charcoal (452 mg) for 2 hr. The catalyst was removed by filtration through a Celite bed. The filtrate was evaporated to dryness and the colorless residue was refluxed with methanol (25 ml) for 10 min, whereupon colorless crystals were formed: yield 1.10 g (80%); mp 209–211° with effervescence; $[\alpha]^{25}D$ +14° (*c* 1.0, H_2O); uv $\lambda_{max}^{pH\ 13}$ 268 mμ (ε 8500); $\lambda_{min}^{pH\ 13}$ 252 mμ (ε 7600); $\lambda_{max}^{pH\ 6.8}$ 267 (ε 8200), 235 (ε 8000) and 198 mμ (ε 21,400); $\lambda_{min}^{pH\ 6.8}$ 252 (ε 7400) and 223 mμ (ε 7600); $\lambda_{max}^{pH\ 2}$ 275 mμ (ε 11,800); $\lambda_{min}^{pH\ 2}$ 239 mμ (ε 2300); ir λ_{max}^{KBr} 2.85 (OH), 3.0 (NH), 6.0, 6.2 (pyrimidine), and 9.3 μ (COC sugar).

Anal. Calcd for $C_{10}H_{16}O_5N_4 \cdot {}^1/_2CH_3OH$: C, 43.75; H, 6.25; N, 19.44. Found: C, 43.52; H, 6.37; N, 19.66.

The nmr spectrum (DMSO-d_6) indicated the presence of 0.5 mol of methanol of crystallization which could not be removed by drying *in vacuo* at 140° for 17 hr.

1-(4-Acetamido-4-deoxy-β-D-glucopyranosyl)cytosine (9).—To a suspension of **7** (3.16 g) in methanol (500 ml) was added acetic anhydride (1.7 ml), and the mixture was stirred at room temperature. The suspension slowly gave a clear solution from which crystals gradually separated. After being stirred continuously overnight, the product was filtered and washed with cold methanol to give **9**: yield 3.20 g (88%); mp 317–319° dec; $[\alpha]^{25}D$ +32° (*c* 1.1, H_2O); uv $\lambda_{max}^{pH\ 13}$ 268 mμ (ε 8400); $\lambda_{min}^{pH\ 13}$ 253 mμ (ε 7500); $\lambda_{max}^{pH\ 6.8}$ 267 (ε 8200), 235 (ε 8000), and 198 mμ (ε 22,000); $\lambda_{min}^{pH\ 6.8}$ 252 (ε 7500) and 222 mμ (ε 7600); $\lambda_{max}^{pH\ 2}$ 276 mμ (ε 12,000); $\lambda_{min}^{pH\ 2}$ 240 mμ (ε 2400); ir λ_{max}^{KBr} 2.78 (OH), 3.07 (NH), 6.02 (C=O *N*-acetyl), 6.02, 6.22 (pyrimidine), 9.1, and 9.55 μ (COC sugar).

Anal. Calcd for $C_{12}H_{18}O_6N_4$: C, 45.86; H, 5.77; N, 17.83. Found: C, 45.61; H, 6.19; N, 17.45.

1-(4-Acetamido-2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucosyl)-N^4-acetylcytosine (8).—A mixture of compound **7** (1.00 g), pyridine (5 ml), and acetic anhydride (5 ml) was refluxed for 30 min. The mixture was evaporated to dryness and the residue was crystallized from ethanol to give **8** as fine needles: yield 1.02 g; mp 293–294° dec; $[\alpha]^{25}D$ +27° (*c* 1.0, DMF); uv λ_{max}^{EtOH} 300 (ε 6000), 249 (ε 15,200), and 210 mμ (ε 16,900); λ_{min}^{EtOH} 276 (ε 4100) and 226 mμ (ε 4800); ir λ_{max}^{KBr} 5.68 (C=O ester), 5.96, 6.1 (pyrimidine), 8.18 (COC ester), 9.28, and 9.48 μ (COC sugar).

Anal. Calcd for $C_{20}H_{26}O_{10}N_4$: C, 49.79; H, 5.39; N, 11.62. Found: C, 49.72; H, 5.38; N, 11.55.

When **8** was treated with methanol containing a catalytic amount of sodium methoxide, compound **9** was obtained in quantitative yield.

Nucleoside **9** (78 mg, obtained from Gougerotin) was suspended in pyridine (1 ml) and the mixture was shaken with acetic anhydride (1 ml). After the mixture had stood for 3 hr, the solvent was removed and the residue was crystallized from ethanol to give **8** as colorless needles: yield 52 mg; mp 294° dec. An ir spectrum of this sample was identical with that of **8** prepared above from **7**.

1-(3-Azido-4-deoxy-6-*O*-trityl-β-D-glucopyranosyl)cytosine (10).—Compound **6** (8.0 g, 0.027 mol) was stirred with trityl chloride (12.0 g, 0.043 mol) in pyridine at 60° for 4 hr and then at room temperature for 15 hr. The mixture was poured onto ice-cold water (800 ml) and stirred for 30 min. The precipitated colorless, shiny crystals were filtered and washed with a small amount of ether to give **10**: yield 14.0 g (97%); mp 221–224° dec.

This compound could be recrystallized from dioxane–ethanol: mp 227–228° dec; uv λ_{max}^{EtOH} 267 mμ (ε 7600); λ_{min}^{EtOH} 275 mμ (ε 1400); $\lambda_{max}^{10\%\ 1\ N\ HCl\ in\ EtOH}$ 272 mμ (ε 13,000).

Anal. Calcd for $C_{29}H_{28}O_5N_6 \cdot H_2O$: C, 62.17; H, 5.37; N, 15.04. Found: C, 62.35; H, 5.39; N, 14.96.

The presence of one molecule of water of crystallization in the analytical sample was confirmed by nmr in DMSO-d_6.

1-(4-Azido-2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-β-D-glucopyranosyl)-N^4-benzoylcytosine (11) and 1-(4-Azido-2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-β-D-glucopyranosyl)dibenzoylcytosine (12).—Compound **10** (13 g, 0.024 mol), previously dried *in vacuo* at 140° overnight, was dissolved in pyridine (200 ml) and treated with benzoyl chloride (13 ml, 0.11 mol) at 5–10° for 4 hr. The mixture was poured onto ice-cold water (1 l.) with vigorous stirring. The semisolid precipitate was treated with boiling ethanol (100 ml) for 10 min and filtered hot. Compound **12** (8.5 g, mp 216–220° with effervescence) was obtained as fine colorless needles, which turned yellow on standing overnight at room temperature in air. One recrystallization of the compound from chloroform–ethanol gave an analytical sample, mp 222° with effervescence. Nmr spectroscopy showed this compound to contain 34–37 aromatic protons. Owing to the insolubility of **11**, it was impossible to determine its uv absorption; it had ir λ_{max}^{KBr} 4.67 (N_3), 5.75 (C=O benzoyl), 6.02, 6.14 (pyrimidine), 6.34, 6.90, 7.92 (COC ester), 9.17, 9.37 (COC sugar), and 14.1 μ (phenyl).

Anal. Calcd for $C_{57}H_{44}O_9N_6$: C, 71.54; H, 4.63; N, 8.78. Found: C, 71.11; H, 4.80; N, 8.82.

The ethanol filtrate was evaporated to dryness. The residue (*ca.* 16 g) was dissolved in a small amount of chloroform, and the solution was diluted with a twofold volume of ether. Colorless needles of **11** crystallized from the mixture: yield 10.5 g; mp 203° with effervescence; uv λ_{max}^{EtOH} 305 (ε 8900), 263 (ε 28,200), and 232 mμ (ε 40,300); λ_{min}^{EtOH} 298 (ε 8300) and 294 mμ (ε 21,700); ir λ_{max}^{KBr} 4.67 (N_3), 5.73 (C=O benzoyl), 5.90, 6.12 (C=O and pyrimidine), 6.75, 7.95 (COC ester), 9.17, 9.37 (COC sugar), and 14.17 μ (phenyl).

Anal. Calcd for $C_{50}H_{40}O_8N_6$: C, 70.42; H, 4.73; N, 9.85. Found: C, 70.29; H, 4.73; N, 9.73.

1-(4-Azido-2,3-di-*O*-benzoyl-4-deoxy-β-D-glucopyranosyl)-N^4-benzoylcytosine (13).—Compound **11** (9.5 g, 0.011 mol) was dissolved in chloroform (100 ml) and the solution was diluted with ethanol (100 ml) containing concentrated hydrochloric acid (0.8 ml). The mixture was stirred for 24 hr at room temperature, then evaporated to dryness *in vacuo* at 30°. The residue, after being dried by azeotropic distillation with benzene, was triturated with ether (100 ml). The solid obtained by trituration was filtered, suspended in ethanol (50 ml), and refluxed for 15 min. The amorphous powder became crystalline during this procedure: yield 4.9 g (72%); mp 229–230° with effervescence; uv λ_{max}^{EtOH} 305 (ε 7700), 263 (ε 24,600), and 231 mμ (ε 37,000); λ_{min}^{EtOH} 292 (ε 7600) and 249 mμ (ε 19,000); ir λ_{max}^{KBr} 2.75 (OH), 3.07 (NH), 5.78 (ester), 5.95 (pyrimidine), 7.92 (COC ester), 9.20, 9.40 (COC sugar), and 14.15 μ (phenyl).

Anal. Calcd for $C_{31}H_{28}O_8N_6$: C, 60.98; H, 4.29; N, 13.76. Found: C, 61.21; H, 4.08; N, 13.99.

1-(4-Azido-4-deoxy-β-D-glucopyranosyluronic acid)cytosine (15).—Compound **13** (2.4 g, 4 mmol) was dissolved in a mixture of pyridine (24 ml) and acetic acid (8 ml). To this was added 16 ml of oxidizing reagent [fourfold excess, prepared by dissolving CrO_3 (6.7 g) in water (10 ml), then diluting to 100 ml with acetic acid]. The mixture was heated at 70° for 24 hr, then poured onto water (400 ml). Crude, blocked uronic acid derivative **14** precipitated and was filtered. This compound possessed a characteristic infrared absorption band at 6.19 μ. Semicrystalline **14** was suspended in methanol (80 ml) and treated with 2 *M* sodium methoxide solution (8 ml) for 17 hr at room temperature. The methanol was evaporated and the residue was partitioned between water (100 ml) and ether (100 ml). The aqueous layer was neutralized with concentrated hydrochloric acid, then extracted with ether (two 100-ml portions). The aqueous layer was passed through a column of Dowex 1 × 8 (OH^-) (100–200 mesh, 4.5 × 20 cm) and the column was washed successively with water (2 l.), 0.02 *N* formic acid (6 l.), and 0.2 *N* formic acid (4 l.). Compound **15** was eluted with 0.2 *N* formic acid. The eluent was evaporated to dryness, and the colorless crystalline residue was suspended in a small amount of water and reevaporated to remove a trace of formic acid. The residue was recrystallized from water to give colorless needles of **15**: mp 260–260.5° with effervescence; yield 704 mg (56.4%); uv $\lambda_{max}^{H_2O}$ 272 mμ (ε 9000); $\lambda_{min}^{H_2O}$ 245 mμ (ε 5800); $\lambda_{max}^{0.1\ N\ HCl}$ 276 mμ (ε 12,200); $\lambda_{min}^{0.1\ N\ HCl}$ 240 mμ (ε 2500); $\lambda_{max}^{0.1\ N\ NaOH}$ 268 (ε 8500) and 237 mμ (ε 8400);

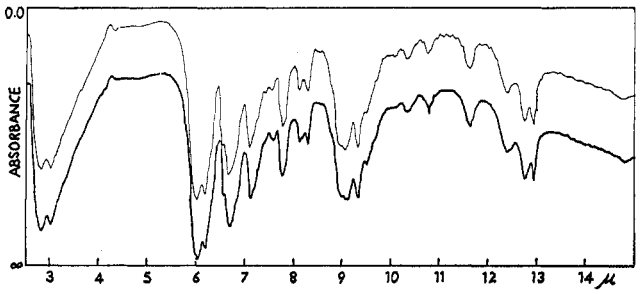


Figure 1.—The infrared spectrum of C-substance. Heavy line = synthetic sample; thin line = sample derived from Gougerotin.

$\lambda_{min}^{0.1\ N\ NaOH}$ 252 (ε 7500) and 225 mμ (ε 8000); ir λ_{max}^{KBr} 2.7, 2.85, 3.35, 4.69, 5.77, 7.01, 7.84, and 9.10 μ.

Anal. Calcd for $C_{10}H_{12}N_6O_6$: C, 38.46; H, 3.87; N, 26.72. Found: C, 38.56; H, 3.81; N, 26.85.

1-(4-Amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine (C-Substance).—The above nucleoside **15** (96 mg) was dissolved in boiling water (20 ml). The solution was cooled to room temperature and then shaken in a hydrogen atmosphere for 2 hr with 10% palladium on charcoal catalyst (49 mg). The catalyst was filtered and the filtrate was evaporated to dryness to give a quantitative yield (88 mg) of synthetic C-substance as colorless, long needles. Recrystallization from water gave an analytical sample with the following properties: mp 235° dec; $[\alpha]^{25}D$ +6° (*c* 0.89, water) [lit.[15] mp 235° dec, $[\alpha]^{20}D$ +2° (water)]. Both the synthetic and the Gougerotin-derived C-substance migrated as a single spot (+6.9 cm) on paper electrophoresis in borate buffer (pH 9.2, 800 V, 4 hr). The infrared spectra of both samples were identical (see Figure 1), as were also their ultraviolet spectral characteristics. A mixture melting (decomposition) point showed no depression.

Anal. Calcd for $C_{10}H_{14}N_4O_6 \cdot {}^1/_2H_2O$: C, 40.68; H, 5.08; N, 18.98. Found: C, 40.70; H, 5.10; N, 18.88.

The nmr spectrum of the analytical sample showed the presence of 0.5 mol of water of crystallization.

Registry No.—**3**, 22176-09-6; **4**, 22176-10-9; **5**, 22176-11-0; **6**, 22176-12-1; **7**, 22212-28-8; **8**, 22176-13-2; **9**, 21209-53-0; **10**, 22176-15-4; **11**, 22176-16-5; **12**, 22176-17-6; **13**, 22176-18-7; **15**, 22176-19-8; C-substance, 22176-20-1.

Acknowledgment.—The authors are indebted to Mr. Marvin J. Olsen for the nmr spectra on a Varian A-60 and to Dr. S. Tatsuoka of Takeda Chemical Industries, Osaka, Japan, for a sample of Gougerotin.

Pyrrolopyrimidine Nucleosides. V. A Study on the Relative Chemical Reactivity of the 5-Cyano Group of the Nucleoside Antibiotic Toyocamycin and Desaminotoyocamycin. The Synthesis of Analogs of Sangivamycin[1]

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Received June 13, 1969

A study of the pyrrolo[2,3-*d*]pyrimidine ring system has revealed that a substituent residing in the pyrimidine ring has a pronounced effect on the reactivity of a cyano group in the pyrrole moiety. It was found that a group (keto) capable of supporting an anion at position 4 decreased the reactivity of the cyano group at position 5 toward nucleophilic attack in comparison to a group (amino) at position 4 incapable of supporting an anion. The reactivity of the cyano groups described above appears to be reversed under acidic conditions. This study has furnished a number of 4,5-disubstituted 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines with carboxamido-type groups (amidoxime, amidrazone, thiocarboxamide) at position 5. These derivatives which contain an amino group at position 4 may be considered as analogs of the nucleoside antibiotic sangivamycin.

A recent investigation[2-4] on the reactivity of the pyrrolo[2,3-*d*]pyrimidine ring system toward electrophilic attack has revealed that the introduction of an electrophile into the pyrrole moiety is dependent to a large extent on the type of substituent already present in the pyrimidine ring. This prompted the present investigation on the possible effect that different substituents in position 4 of the pyrrolo[2,3-*d*]pyrimidine ring might have on the chemical reactivity of a cyano group at position 5. This study was also of considerable interest, since, although both groups are in different rings, they lie in very close proximity.

Toyocamycin[5] [**5**, 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine] has an exocyclic amino group at position 4 and a cyano group in the adjacent ring at position 5.

Treatment of toyocamycin (**5**) (Scheme I) with hydrazine at reflux temperature for 2 hr furnished a good yield of 4-amino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine-5-carboxamidrazone (**7**) as established by pmr, ir, and uv (Table I) spectra and elemental analysis. Therefore, nucleophilic attack at the 5-cyano group had proceeded in the presence of an amino group at position 4, which indicated that the cyano group of toyocamycin was susceptible to nucleophilic attack. This was corroborated by the formation of 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidoxime (**4**) in 85% yield from **5** on treatment with hydroxylamine. However, the conversion of **5** into 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamidine (**2**) with methanolic ammonia was unsuccessful even under drastic conditions. This established that, although the cyano group of **5** was suscep-

(1) This investigation was supported by Research Contract PH-43-65-1041 with Chemotherapy, National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) Part of this work has been presented in a preliminary report: L. B. Townsend, B. C. Hinshaw, R. L. Tolman, R. K. Robins, and J. F. Gerster, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., 1968, Abstract MEDI 29.

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